



## Full Length Article

# Evaluation and use of a serological assay for the detection of antibodies to *Lawsonia intracellularis* in swine



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Received 8 June 2014; revised 27 July 2014; accepted 19 August 2014

Available online 3 December 2014

## KEYWORDS

Enzyme linked immunosorbent assay ELISA;  
Antibodies;  
Immuno-peroxidase monolayer assay;  
IPMA;  
Swine;  
Ileitis;  
*Lawsonia intracellularis*

**Abstract** Porcine proliferative enteropathy (PPE) is a common and economically important gastro-intestinal disease of swine caused by the intracellular bacterium, *Lawsonia intracellularis*. Conventional tests to detect antibody responses to *L. intracellularis* include the immuno-peroxidase monolayer assay (IPMA), immuno-fluorescent antibody test (IFAT) and a lipopolysaccharide ELISA (LPS-ELISA). These tests are not commercially available. Therefore, objective of this study is to evaluate the performance of a commercial *L. intracellularis* blocking ELISA. Performance of the commercial ELISA was compared to the IPMA and LPS-ELISA using serum from experimentally infected animals ( $N = 40$ ). The prevalence of *L. intracellularis* sero-positive animals was assessed by comparing suspect and randomly selected sera ( $N = 394$ ). The commercial ELISA, IPMA and a non-commercial lipopolysaccharide (LPS) LPS-ELISA showed a 95% correlation when tested using experimentally derived known status samples. When compared to the IPMA the sensitivity of the commercial ELISA was 91% while the specificity was 100%. Therefore, the diagnostic sensitivity and specificity of the commercial *L. intracellularis* ELISA was comparable to the LPS-ELISA and IPMA. A comparison of suspect and randomly selected field samples with the commercial ELISA indicated that *L. intracellularis* sero-positivity is widespread and does not correlate with possible disease status.

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**Abbreviations:** PPE, porcine proliferative enteropathy; IFAT, immuno-fluorescent antibody test; IPMA, immuno-peroxidase monolayer assay; ELISA, enzyme linked immunosorbent assay; LPS, lipopolysaccharide; IHC, immunohistochemistry; PCR, polymerase chain reaction

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Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.

<http://dx.doi.org/10.1016/j.ijvsm.2014.08.001>

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## 1. Introduction

Porcine proliferative enteropathy (PPE), also known as ileitis, is an important production disease of growing piglets. The etiological agent of PPE is *Lawsonia intracellularis*, an obligate intracellular bacterium that colonizes the intestinal epithelial cells leading to thickening and proliferation of crypt cells. Therefore, characteristic histopathology consists of the presence of proliferative lesions in the mucosa of the small and large intestine [1–3]. Growing piglets are commonly affected by PPE. Naïve adults experience the acute form of the disease. PPE is characterized by brownish diarrhea, loss of condition and poor weight gain resulting in significant economic losses [4,5]. While the exact estimates for the sero-positivity of *L. intracellularis* in swine production areas in the U.S. are not available, PPE is present worldwide in pork production systems [4,6].

Immunohistochemistry (IHC) is a routine procedure for post-mortem diagnosis of PPE [7]. Polymerase chain reaction (PCR) based tests are available for the detection of *L. intracellularis* in fecal material. However, these tests are relatively more expensive than serology and have variable sensitivity [8–10]. With the introduction of an attenuated, live porcine ileitis vaccine in the U.S. market [11], ante-mortem detection and diagnosis of PPE, particularly by serology, has gained practical utility. Serology is an important tool in understanding the infection kinetics in herds. Seroprofiles are necessary to better position vaccination and/or medication strategies in herds with proliferative enteropathy problems as the optimal time for vaccine placement is after the maternal antibodies have waned [12].

Traditional culture methods for *L. intracellularis* are cumbersome and time-consuming. Yet, an indirect immunofluorescence assay (IFA) or immuno-peroxidase monolayer assays (IPMA) were the only available tests [7,13,14] in diagnostic laboratories to detect antibody responses to *L. intracellularis* until a lipopolysaccharide (LPS) based ELISA was developed (LPS-ELISA). However, the LPS-ELISA is not commercially available [15]. A commercial monoclonal antibody based blocking ELISA became available a few years ago [16]. The manufacturer-claimed performance metrics for this assay included a sensitivity of 96.5% and specificity of 98.7%. However, only one report comparing the performance of the commercial ELISA to the IFAT, which is not widely available, is published so far [17]. Therefore, in this study we have evaluated the performance of the commercial ELISA by comparing its performance with assays such as the IPMA and LPS ELISA.

## 2. Materials and methods

### 2.1. Serum samples for assay verification

Forty swine sera of known status collected from experimentally infected animals were provided by Boehringer Ingelheim Vetmedica, Inc. (BIVI). Twenty of these were collected from infected animals while the other twenty were from uninfected controls. Post-infection antibody responses were confirmed by IFAT as described by Kroll et al., [15]. To determine if the presence of clinical signs correlated with sero-positivity, a total of 308 field samples derived from eight case submissions to the Iowa State University Veterinary Diagnostic laboratory were tested by the commercial ELISA (bioScreen Ileitis Antibody ELISA, Synbiotics Corporation, Lyon, France).

The selected cases were suspect for ileitis due to a case history of diarrhea and loss of condition in the affected animals. To determine the general population prevalence, 86 other field samples were blinded to case history and randomly selected for analysis by the commercial ELISA.

### 2.2. Comparison of the performance of the commercial ELISA, IPMA and LPS ELISA

Antibodies against *L. intracellularis* in the experimental, known-status samples were detected by two independent diagnostic laboratories either by an LPS based ELISA [15] or an IPMA [13,14]. Inter-assay variation for the commercial ELISA was measured by three independent assessments of the experimental known status samples. To determine whether the rate of sero-positivity correlated with possible disease status, suspect and random field samples were evaluated by the commercial ELISA.

### 2.3. Commercial *L. intracellularis* ELISA

Testing with the commercial ELISA kit (bioScreen Ileitis Antibody ELISA, Synbiotics Corporation, Lyon, France) was carried out following manufacturer's instructions. The commercial ELISA kit contains *L. intracellularis* antigen which is immobilized on a 96 well ELISA plate. Following the kit protocol, swine serum or plasma samples are placed in the wells. Following incubation and wash steps, a monoclonal antibody conjugated to peroxidase specific to an *L. intracellularis* epitope, is added to the wells. If the sample contains anti-*L. intracellularis* antibodies, the antigenic sites are blocked thus preventing binding of the monoclonal antibody conjugate. Therefore, color development is either very low or absent. If the sample does not contain anti-*L. intracellularis* antibodies, the antigen is free to bind to the monoclonal antibody resulting in intense color development. The optical density is measured at 450 nm in a microplate reader and the percent inhibition (PI) of the positive controls and test samples relative to the negative controls is calculated. Any serum sample presenting a PI of  $\geq 30\%$  is considered positive. Any samples presenting a PI of  $\leq 20\%$  are considered negative. Samples within 20–30% range are considered suspects.

### 2.4. Statistical analysis

For the experimental samples, the agreement between the commercial ELISA and the LPS based ELISA was assessed by the Pearson's correlation coefficient with a raw continuous scale using the PI values. The dichotomous agreement between the commercial ELISA, the LPS based ELISA and the IPMA was measured by kappa coefficients. The sensitivity, specificity, positive and negative predictive values of the commercial ELISA was determined by standard formulae.

## 3. Results

### 3.1. Comparative detection of known-status samples on the commercial ELISA, LPS ELISA and IPMA

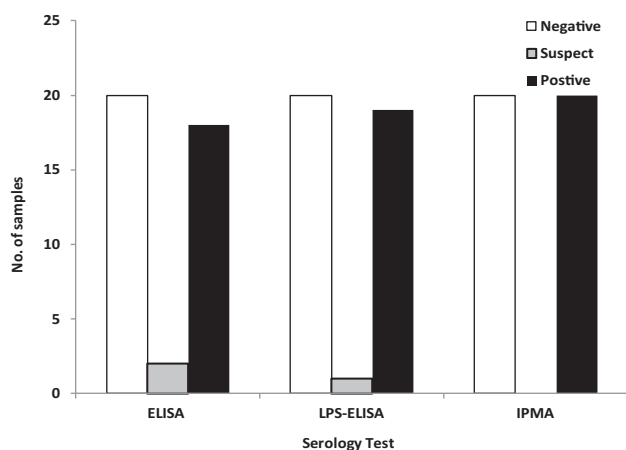
All of the twenty known status negative samples were negative when tested with the commercial ELISA, LPS ELISA and

IPMA. Of the twenty known positive samples, all twenty were positive using IPMA while, nineteen were positive with the LPS ELISA and 18 were positive using the commercial ELISA. Two known-status positive samples showed a variable reaction on the commercial ELISA with one sample being negative on two runs and suspect on the third. This sample was also negative on the LPS ELISA while it was positive on the IPMA. The second sample was negative on two runs, positive on the third and positive on both the LPS ELISA and IPMA. Both of these samples had PI values that were either high negatives or low positives and therefore could be considered 'grey zone' samples which are neither clearly positive nor negative at the time of collection, probably because of ongoing sero-conversion in the host (Fig. 1).

The commercial ELISA showed consistent performance and very little intra-assay variation when the PI values over three independent assays was compared as Pearson's correlation coefficients. Similarly, the average correlation coefficient between the commercial ELISA and the LPS ELISA was 0.92 (Table 1). When the results were compared between the three assays as kappa coefficients, the lowest agreement between the three replicate values on the commercial ELISA and between the three tests was 0.95, indicating that all three tests were comparable to each other in performance (Table 2).

### 3.2. Sensitivity and specificity assessment

As all the known status samples were correctly identified by the IPMA, its accuracy, sensitivity and specificity was 100%. When the IPMA was used as the comparative test, the sensitivity of the commercial ELISA was 91% while the specificity was 100%. The sensitivity of the LPS ELISA was 95% while the specificity was 100%. The LPS ELISA was 97.5% accurate. Therefore, the positive predictive values for all three tests were 100% while the negative predictive values for the commercial ELISA was 90% and 95% for the LPS ELISA respectively.



**Figure 1** Comparison of the detection of experimentally derived, samples of known status by three different serological tests for *L. intracellularis*. Twenty known positive and twenty known negative samples were assessed on either a commercial ELISA, an LPS based ELISA or IPMA. The graph shows results as the number of positive, suspect and negative samples. The correct status of 95% of samples was detected consistently by all three tests with the IPMA showing 100% accuracy.

### 3.3. Assessment of field samples by the commercial ELISA and IPMA

Forty-seven percent of the samples from the *L. intracellularis* suspect cases were positive on the commercial ELISA while 44% were negative and 9% were suspect (Table 3). Among the randomly selected samples approximately 66% were positive, 26% were negative and 8% were suspect (Table 3). Only one case from each data-set was completely negative.

## 4. Discussion

Ante-mortem diagnosis of PPE has become increasingly important in the prevention and control of this economically important disease. Molecular methods such as PCR assays are available. A real-time PCR assay had a sensitivity of only 0.84 and specificity of 0.93 in detecting *L. intracellularis* infected swine feces. Therefore PCRs are not only less sensitive, expensive and require specialized facilities and equipment [7,10,18] but also have little value in predicting the optimal time for vaccine placement. Serological methods are relatively inexpensive and user-friendly and therefore are the first method of choice in screening swine herds for antibodies against *L. intracellularis*.

The IPMA is the gold standard for serological diagnosis of PPE but plate preparation is cumbersome and the procedure has subjectivity. ELISA test formats have the greatest ease of use and high throughput required for herd screening [15,19]. With the recent availability of a commercial ELISA a re-evaluation and comparison of assay performance between previously available assays and the commercial assay is important to ensure accurate serological diagnosis of PPE.

In this study three successive runs of experimentally derived, samples of known status resulted in excellent repeatability with the commercial ELISA. In the assessment with the experimental samples the IPMA was marginally more sensitive than both of the ELISA's tested as all known status samples were correctly detected as either positive or negative by this test (Fig. 1, Table 1). The commercial ELISA and the LPS-ELISA were slightly less sensitive in detecting 'grey zone' samples (91% and 95%, respectively) but did not detect false positives. These results were in agreement with findings by others showing that the IPMA is highly specific and sensitive [13,14]. As the immunofluorescence antibody test (IFAT) is comparatively less specific and prone to false positive results and less commonly available, this method was not included for assessment in this study. Moreover, the performance of the commercial ELISA has been compared to the IleITest IFAT (Elanco Animal Health, Indianapolis, Indiana, USA) [17]. In this study, when compared to the IFAT, the sensitivity of the blocking ELISA was 72%, specificity was 93%, the positive predictive value was 0.82 and the negative predictive value was 0.89.

The findings of the current study that the LPS ELISA was 95% sensitive while the specificity was 100% and the accuracy was 97.5% was a slightly lower estimation when compared to the original study which showed a 99.5% sensitivity, probable due to the smaller number of known status samples used [15]. Although the LPS ELISA is a useful serological tool in early detection of *L. intracellularis* in swine, the need for purified antigen make the cost of the ELISA prohibitive. In contrast, the IPMA showed a higher sensitivity and specificity of

**Table 1** Performance of the commercial enzyme linked immunosorbent assay (ELISA)\*.

	ELISA 1st replicate <sup>†</sup>	ELISA 2nd replicate <sup>†</sup>	ELISA 3rd replicate <sup>†</sup>	LPS-ELISA <sup>†</sup>
1st replicate <sup>†</sup>	1.00	0.99	0.98	0.91
2nd replicate <sup>†</sup>	0.99	1.00	0.97	0.90
3rd replicate <sup>†</sup>	0.98	0.97	1.00	0.95
LPS-ELISA <sup>†</sup>	0.91	0.91	0.95	1.00

\* ELISA – commercial ELISA, LPS-ELISA – lipopolysaccharide ELISA.

† Pearson correlation coefficients with a sample size  $N = 40$ .

**Table 2** Assessment of agreement as kappa coefficients for the *Lawsonia intracellularis* serological tests\*.

	ELISA 1st replicate <sup>†</sup>	ELISA 2nd replicate <sup>†</sup>	ELISA 3rd replicate <sup>†</sup>	LPS-ELISA <sup>†</sup>	IPMA <sup>†</sup>
ELISA	1	0.975	0.975	0.975	0.950
1st replicate	1	0.949	0.949	0.949	0.900
ELISA		0.975	0.975	0.975	0.950
2nd replicate		0.949	0.949	0.949	0.900
ELISA			1	1	0.975
3rd replicate			1	1	0.950
LPS-ELISA					0.975
					0.950

\* Commercial ELISA, LPS-ELISA – lipopolysaccharide ELISA, IPMA – immuno-peroxidase monolayer assay.

† Agreement percentage of dichotomous values derived from three commercial ELISA replicates, the LPS ELISA and the IPMA, kappa coefficients with a sample size of  $N = 40$ .

**Table 3** Percentage of sero-positive animals in eight diagnostic cases suspect for *Lawsonia intracellularis* ( $N = 308$ ) as well as six randomly selected cases blinded to case history ( $N = 86$ )\*.

Case type	Positives	Negatives	Suspects
D-1	5/50 (10%)	40/50 (80%)	5/50 (10%)
D-2	88/180 (49%)	70/180 (39%)	22/180 (12%)
D-3	10/10 (100%)	0/10 (0%)	0/10 (0%)
D-4	0/10 (0%)	10/10 (100%)	0/10 (0%)
D-5	15/30 (50%)	14/30 (47%)	1/30 (3%)
D-6	10/10 (100%)	0/10 (0%)	0/10 (0%)
D-7	10/10 (100%)	0/10 (0%)	0/10 (0%)
D-8	6/8 (75%)	1/8 (12.5%)	1/8 (12.5%)
Total – D	144/308 (47%)	135/308 (44%)	29/308 (9%)
R-1	7/10 (70%)	0/10 (0.00%)	3/10 (30%)
R-2	0/18 (0.00%)	18/18 (100%)	0/18 (0.00%)
R-3	11/19 (58%)	4/19 (21%)	4/19 (21%)
R-4	29/29 (100%)	0/29 (0.00%)	0/29 (0.00%)
R-5	1/1 (100%)	0/1 (0.00%)	0/1 (0.00%)
R-6	9/9 (100%)	0/9 (0%)	0/9 (0%)
Total – R	57/86 (66.28%)	22/86 (25.58%)	7/86 (8.14%)

\* Diagnostic cases suspected to involve *Lawsonia intracellularis* shown as D-1, D-2 and so on. Random cases with blinded history shown as R-1, R-2 and so on.

100% compared to 91% and 100%, respectively in the original study [13]. The commercial ELISA has a manufacturer claimed sensitivity and specificity of 96.5 and 98.7% [16]. However, the current study found that the sensitivity was slightly lower at 91% and the specificity was higher at 100% with experimentally derived samples of known status and higher than the values reported by Jacobson et al.'s comparison to the IFAT [17].

In the assessment of field samples, a majority of the herds examined were sero-positive for *L. intracellularis*. Surprisingly, there were no significant differences between samples from PPE-suspect cases and randomly assigned samples which represented the baseline population in our study. A possible reason for the lower sero-prevalence levels in the suspect samples when compared to the random samples is that the clinical signs of enteritis in the suspect samples could have been caused by other common agents such as the transmissible gastro-enteritis virus, rota virus or due to nutritional and metabolic reasons. The findings in this study also indicate that sero-prevalence is widespread and unless a quantitative assessment is carried out by testing paired samples, ELISA results cannot be used as an indicator of disease status. Although the current study was not specifically designed to determine the rates of sero-prevalence in the region, it would have been enhanced by correlating age with sero-conversion in positive herds. However, the study of these parameters is beyond the scope of this study.

## 5. Funding

The author(s) received no external financial support for the research, authorship, and/or publication of this article. The study was funded by the Department of Diagnostic and Production Animal Medicine, Iowa State University and publication by the Veterinary and Microbiological Sciences Department, N. Dakota State University.

## Acknowledgements

We would like to acknowledge the technical help rendered by Ms. Kathy Lin and the staff of the Iowa State University Veterinary Diagnostic Laboratory.



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